COMPOSITIONS AND METHODS FOR SILENCING EBOLA VIRUS GENE EXPRESSION

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application claims priority to U.S. Provisional Application No. 61/226,959, filed Jul. 20, 2009, and U.S. Provisional Application No. 61/286,741, filed Dec. 15, 2009, the disclosures of which are hereby incorporated by reference in their entirety for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with Government support under Project No. 04-4-7J-012, awarded by the Defense Threat Reduction Agency. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Filoviruses (e.g., Ebola virus (EBOV) and Marburg virus (MARV)) are among the most lethal and destructive viruses. They cause severe, often fatal viral hemorraghic fevers in humans and nonhuman primates (e.g., monkeys, gorillas, and chimpanzees). Filoviruses are of particular concern as possible biological weapons since they have the potential for aerosol dissemination and weaponization.

The incubation period for Filovirus infection ranges from 2 to 21 days. The onset of illness is abrupt and is characterized by high fever, headaches, joint and muscle aches, sore throat, fatigue, diarrhea, vomiting, and stomach pain. A rash, red eyes, hiccups and internal and external bleeding may be seen in some patients. Within one week of becoming infected with the virus, most patients experience chest pains and multiple organ failure, go into shock, and die. Some patients also experience blindness and extensive bleeding before dying.

Filoviridae are a family of RNA viruses. Two members of the Filoviridae family have been identified: EBOV and MARV. There is one identified strain of MARV and four identified subtypes (i.e., strains) of EBOV: Ebola-Zaire, Ebola-Sudan, Ebola-Ivory Coast (i.e., Ebola-Tai), and Ebola-Reston. The exact origin, locations, and natural habitat of Filoviridae are unknown. However, on the basis of available evidence and the nature of similar viruses, it is postulated that Filoviridae are zoonotic (i.e., animal-borne) and are normally maintained in an animal host that is native to the African continent.

For more than 30 years, EBOV has been associated with periodic episodes of hemorrhagic fever in Central Africa that produce severe disease in infected patients. Mortality rates in outbreaks have ranged from 50% for the Sudan species of EBOV (SEBOV) to up to 90% for the Zaire species of EBOV (ZEBOV) (Sanchez et al., Filoviridae: Marburg and Ebola Viruses, in *Fields Virology* (eds. Knipe, D. M. & Howley, P. M.) 1409-1448 (Lippincott Williams & Wilkins, Philadelphia)). An outbreak late in 2007 caused by an apparently new species of EBOV in Uganda resulted in a fatality rate of about 25% (Towner et al., *PLoS Pathog.,* 4:e1000212 (2008)). ZEBOV has also decimated populations of wild apes in this same region of Africa (Walsh et al., *Nature,* 422:611-614 (2003)).

Prevention and treatment of EBOV infections presents many challenges. In fact, there are no vaccines or postexposure treatment modalities available for preventing or managing EBOV infections. Patients instead receive supportive therapy, i.e., electrolyte and fluid balancing, oxygen, blood pressure maintenance, and treatment for any secondary infections.

Thus, there is a need for compositions and methods for treating and preventing EBOV infections, e.g., by specifically modulating EBOV gene expression. The present invention addresses these and other needs.

BRIEF SUMMARY OF THE INVENTION

The present invention provides compositions comprising therapeutic nucleic acids (e.g., interfering RNA such as siRNA) that target Ebola virus (EBOV) gene expression and methods of using such compositions to silence EBOV gene expression. More particularly, the invention provides unmodified and chemically modified interfering RNA (e.g., siRNA) which silence EBOV gene expression and methods of use thereof, e.g., for preventing or treating EBOV infections caused by one or more EBOV species such as Zaire EBOV. The invention also provides serum-stable nucleic acid-lipid particles (e.g., SNALP) comprising interfering RNA (e.g., siRNA), a cationic lipid, and a non-cationic lipid, which can further comprise a conjugated lipid that inhibits aggregation of particles. Methods of silencing EBOV gene expression by administering interfering RNA (e.g., siRNA) to a mammalian subject are also provided.

As explained herein, it has surprisingly been found that the SNALP formulations of the present invention containing a combination of interfering RNA (e.g., siRNA) molecules targeting at least two or all three of the EBOV L-pol, VP24, and VP35 genes were capable of providing complete postexposure protection of nonhuman primates against a lethal EBOV challenge. In particular embodiments, the SNALP formulations described herein comprising a cocktail of interfering RNAs (e.g., siRNAs) targeting any combination of at least two (or all three) of the EBOV L-pol, VP24, and VP35 genes demonstrate an increased potency (i.e., increased silencing activity) and/or an increased tolerability (e.g., a more favorable toxicity profile), e.g., when compared to other nucleic acid-lipid particle compositions previously described.

In one aspect, the present invention provides interfering RNA molecules such as siRNA that target EBOV L-polymerase (L-pol), VP24, VP30, VP35, VP40, nucleoprotein (NP), and/or glycoprotein (GP) expression. The interfering RNA (e.g., siRNA) molecules of the invention are capable of inactivating EBOV and/or inhibiting the replication of EBOV in vitro or in vivo.

In certain embodiments, the interfering RNA comprises at least one or a cocktail (e.g., at least two, three, four, five, six, seven, eight, nine, ten, or more) of unmodified and/or modified interfering RNA (e.g., siRNA) sequences that silence EBOV gene expression. In some instances, the cocktail of interfering RNA (e.g., siRNA) molecules may comprise sequences which target the same region of the EBOV genome. In other instances, the cocktail of interfering RNA (e.g., siRNA) molecules may comprise sequences which target different regions of the EBOV genome. In further instances, the cocktail of interfering RNA (e.g., siRNA) molecules may comprise sequences which target different EBOV species or subtypes. In certain instances, one or more (e.g., at least two, three, four, five, six, seven, eight, nine, ten, or more) modified interfering RNA (e.g., siRNA) that silence EBOV gene expression are present in a cocktail with one or more (e.g., at least two, three, four, five, six, seven, eight, nine, ten,